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(54) Title: ENTERAL VACCINE FOR VAGINAL INFECTIONS

(57) Abstract

An enteral non-adjuvanted vaccine comprises a killed microorganism which infects the vagina. The microorganism may be a fungus, such as *Candida albicans*, a bacterium, such as *Gardnerella vaginalis* or *Neisseria gonorrhoea*, a protozoon, such as *Trichomonas vaginalis*, or a virus, such as *Herpes genitalis*. The absence of adjuvant gives a significant improvement in clearance of the microorganisms, compared to adjuvanted compositions.

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ENTERAL VACCINE FOR VAGINAL INFECTIONS

This invention relates to a vaccine for protecting against fungal, bacterial or protozoal infections of the vagina.

The aetiology of vaginitis, or inflammation of the vagina, is frequently microbial, particularly fungal, bacterial or protozoal. A common fungal cause of vaginitis is caused by the monilia *Candida albicans*, which results in the condition known as thrush. It is estimated that approximately 7% of all adult women are liable to candidiasis. At their least, the symptoms are uncomfortable, and in some cases the patient is virtually unable to lead a normal life.

Among bacterial causes of vaginal infection are *Gardnerella vaginalis* and the gonococcus *Neisseria gonorrhoea*. The consequences of gonorrhoeal infection can be serious: not only is the acute stage of infection painful, the chronic effects can include prolonged ill health and sterility or recurring miscarriages.

The protozoon *Trichomonas vaginalis* is present in the vagina of about 30 to 40% of women. Although often non-pathogenic, it sometimes becomes so and causes inflammation of the genital passages, with vaginal discharge.

A viral cause of vaginalis is the virus *Herpes genitalis*.

Systemic and/or local treatment of women with recurrent infection is disappointing, at least for *C. albicans*: None of the available therapeutic regimes reduces the frequency of attack.

There is clearly a need for effective vaccines against these infections, and it is to this need that the present invention is directed. In the development of an enteric vaccine against microbes which infect the vagina, it has been found that the presence of an adjuvant has not had the expected beneficial effect in enhancing the immune response. What was in fact found was that adjuvant either delayed or prevented clearance of the infection.

According to a first aspect of the invention, there is provided an enteral non-adjuvanted vaccine comprising a killed microorganism which infects the vagina.

A prior example of an non-adjuvanted oral vaccine is disclosed in WO-A-8605691. This prior application discloses an enteral non-adjuvanted monobacterial vaccine comprising killed *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* or *Staphylococcus aureus* bacteria for the purpose of preventing acute bronchitis in patients having chronic mucosal inflammation. Apart from the major distinction that WO-A-8605691 is primarily concerned with the bronchial tract and the present invention is exclusively concerned with infections of the vagina, the reason for the infection in each case is rather different. In WO-A-8605691, the reason for the excess of pathological organisms in the bronchia is damage to, and obstruction of, the airways; in the case of vaginal infections, it seems that infection arises when the pool of effector T-cells is depleted, which may be a condition in a significant proportion of women. Evidence for this comes from direct positive skin tests to *Candida* and the observed reversal with T-cell expansion treatment.

In studies relating to the work disclosed in WO-A-8605961, tests were carried out on normal individuals, some or whom had, and some of whom did not have, antibody to the bacteria in question; those with antibody did not stimulate further antibody on subsequent challenge, whereas those without did. That was the basis for the teaching of WO-A-8605961 which disclosed the omission of any adjuvant component, which would be expected to enhance antibody production. In contrast, in experiments leading up to the present invention, even immunologically naive experimental animals failed to generate antibodies when challenged with *C. albicans*; and it was found that the presence of the adjuvant cholera toxin, known to be a potent mucosal antibody stimulant, reduced the protection conferred by immunogenic killed organisms, probably reflecting blocking antibody or immune deviation. The mechanisms at work therefore do not seem to be parallel.

Vaccines of the present invention are suitable for enteral administration. While rectal administration may be preferred in some cases, orally administrable formulations will usually be those of choice.

The enteric vaccine may be in the form of tablets, especially enteric coated tablets, granules, capsules or dragees for oral administration, or provided eg as suppositories for rectal administration. The dosage unit form may contain from approximately 10^9 microorganisms to approximately 10^{13} microorganisms preferably from approximately 10^{10} microorganisms to approximately 10^{12} microorganisms, together with suitable carriers of organic or inorganic nature.

One or more suitable carriers may be included. They include: fillers, such as sugars (for example lactose, saccharose, mannitol or sorbitol), cellulose preparations, calcium phosphates, (for example calcium phosphate or calcium hydrogen phosphate) and amino acids (for example glycine); binders, such as starch pastes (for example, based on corn, wheat, rice or potato starch), gelatine, gums (such as tragacanth), methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; and/or, if desired, disintegrators, such as the above-mentioned starches, carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar and/or alginic acid or a salt thereof, such as sodium alginate. Other ingredients may include: flow-regulating agents and lubricants, for example silica, talc and stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol; and/or further choleretic agents, such as sodium taurocholate, sodium tauroglycocholate or ox bile. For dragée cores, there may be used, *inter alia*, concentrated sugar solutions which may contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide; they may be coated with solutions of lacquer in suitable organic solvents or solvent mixtures or, for the production of coatings that are resistant to gastric juices, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Dyes or pigments can be added to the tablets or dragée coatings, for example for identification purposes or to indicate different doses of active ingredient. Compounds eliciting an antigenic response, that is to say adjuvants, are excluded as carriers or adjuncts in the vaccine of the invention.

A particular feature of vaccines of the present invention is that no adjuvant is added to the killed microorganisms; the vaccine stimulates only a limited antigenic response. Adjuvants usually added to vaccines and absent in the vaccine of the present invention include cholera toxin, killed bacteria known to illicit a strong antigenic response, for example killed *Mycobacterium tuberculosis* such as *Bacillus Calmette Guerin* (BCG) or killed *Corynebacterium paryum*. Each species acts as an adjuvant for the other bacteria. Other common adjuvants include inorganic polymeric material, such as aluminium oxide or aluminium phosphate, which is especially useful in parenteral vaccines.

It is preferred that a vaccine of the invention contain only a single species of microorganism, to avoid the possibility of one microorganism adjuvanting another. However, two or more different microorganisms may be present when there is little or no adjuvanting effect between them.

A vaccine of the present invention comprises a killed microorganism which infects the vagina. The microorganism, which may be killed in any convenient way, for example by the application of formalin, may be fungal, bacterial, protozoal or viral. Fungal organisms infecting the vagina include the monilia *Candida albicans*; bacterial vaginal infections can be caused by the gonococcus *Neisseria gonorrhoea* or *Gardnerella vaginalis*; protozoal infections may be caused by *Trichomonas vaginalis*; and viral infections may be caused by herpes viruses, particularly *Herpes genitalis*.

While it is likely that the prevention of infection by *C. albicans* will be one of the primary aims of the

invention, and indeed it is on *C. albicans* that the experimental work below is focused, it is reasonable to expect that infections by other microorganisms can be prevented because it has been shown in other model systems that the major effectors for clearance of the infecting *C. albicans* microorganisms are T-lymphocytes. T cells would be expected to act on other mucosally mediated microorganisms such as other fungi, bacteria and protozoa as well as on neurally mediated viruses such as herpes.

The invention may have veterinary applications, although it is to the prevention of disease in humans that the invention is particularly addressed.

As will be discussed below, killed microorganisms in a vaccine of the invention can be administered in a high oestrogen environment. ("Oestrogen" is used in this specification as a generic term to cover compounds with oestrogenic activity; oestrogen itself is included, as are its various derivatives and analogues which retain its activity). It is possible, although not always optimal, for oestrogen (at least in an orally administrable form) to be co-formulated with the killed microorganisms in the vaccine of the invention.

According to a second aspect of the invention, there is provided a process for the preparation of a vaccine as described above, the process comprising admixing killed microorganisms with a pharmaceutically or veterinarily acceptable carrier, filler, diluent and/or other adjunct.

Some such processes for the manufacture of vaccines are known in the art. Bacteria and certain other microorganisms may be grown on plates, eg agar plates

containing various nutrients, or in suspension in fermentation broth containing nutrients in dissolved form (such as milk hydrolysates, lactalbumin hydrolysates, corn steep liquors, glucose, starches and tryptic soy broth) and growth stimulating substances (such as hormones and coenzymes), or in the form of serum dilutions (for example of horse serum or foetal calf serum). The cultures must be kept under strictly aseptic conditions, and optionally small amounts of antimicrobials, such as antibiotics, are added to prevent overgrowth by unwanted organisms.

The organisms are killed for example with formalin, phenol or other, optionally homogenised and washed extensively. Sterility of the killed organisms has to be tested carefully, eg by inoculation into a medium or an agar plate known to allow rapid growth of viable organisms.

Pharmaceutical preparations for oral or rectal administration can be obtained from the killed organisms by conventional lyophilising, drying, mixing, granulating and/or confectioning processes under sterile conditions.

The invention is useful in a method of preventing vaginal infections in humans or non-human animals, the method comprising enterally administering a vaccine as described above. According to a third aspect of the invention, therefore, there is provided the use of a killed microorganism which infects the vagina in the preparation of an enteral adjuvant-free vaccine.

Preferably one to three unit doses of the vaccine containing 10^{10} to 10^{12} killed bacteria are administered for five to seven consecutive days. More than one

course may be required, and three courses at approximately three to five week intervals are preferred.

It appears that vaccines of the invention are particularly effective when administered in the presence of oestrogen. It is thought that oestrogen serves *in vivo* to control a "gate", which enables cytotoxic T cells to reach the vaginal mucosa. Evidence for this comes by analogy from observations on B cells, and clinical experience shows that many women tend to suffer an episode of candidiasis shortly before their period is due, that is to say in a period of oestrogen deficiency.

In women not taking oestrogen-based contraceptives, it is therefore appropriate to administer the vaccine post-menstruation in the first half of the menstrual cycle, for example beginning at day 5 or day 7 after the beginning of the period. Women taking a oestrogen-based contraceptive may take the vaccine of the invention at any time other than the week of their period. It is possible for oestrogen to be administered simultaneously or sequentially with vaccine of the invention.

According to a fourth aspect of the invention, there is provided a product comprising a vaccine as described above and a compound having oestrogenic activity for combined, simultaneous or sequential administration in the prevention of microbial infections of the vagina.

Preferred features for each aspect of the invention are as for each of the other aspects, *mutatis mutandis*.

The invention will now be described with reference to the following examples.

EXAMPLE 1

Protection of Mice Against *Candida albicans* Intravaginal Challenge by Adjuvant-Free Oral Vaccine

The experimental animals used in this and subsequent examples were Balb/C mice, used in groups of five. The immunising and infecting organism was a strain of *Candida albicans* isolated from the human patient with recurrent vulvo-vaginal candidiasis selected because it returned the highest titre from an intravaginal wash. To prepare the vaccine, *C. albicans* was killed at the blastocondia stage by the application of formalin.

Each of the experimental mice received 10^8 killed *C. albicans* orally in bicarbonate buffer on days 0, 2, 4, 6, 14 and 21. Each of the control group received bicarbonate buffer only, on the same days.

On day 25, each mouse received 100 µl of oestrogen subcutaneously and, on day 28, 20 µl of a suspension of 5 x 10^5 blastocondia stage *C. albicans* cells were introduced vaginally. At day 35, and at seven day intervals thereafter, 100 µl of oestrogen was again administered subcutaneously.

Also at day 35, and at seven day intervals thereafter, a vaginal wash was taken, and the number of viable *Candida* cells were counted and recorded as the number of organisms per cubic millimetre. There results are shown in the following table.

	Day 35	Day 42	Day 49
Control	275	106	157
Vaccine	82	35	0

The results show a significant, and indeed startling, decrease in the number of viable candida cells within three weeks of challenge. In contrast, the control group showed no such significant decrease. Although some decrease was reported, this was because of a difficulty with one of the control mice, whose count was arbitrarily scored as zero.

COMPARATIVE EXAMPLE 1

Administration in the Presence of Adjuvant

The procedure of Example 1 was repeated, except that the adjuvant cholera toxin (10 μg) was added to the *C. albicans*/bicarbonate buffer formulation on days 0, 14 and 21. The results are shown in the following table.

	Day 35	Day 42	Day 49
Adjuvant Vaccine	623	217	77

It can therefore be seen that the addition of cholera toxin causes an initial *increase* in the growth of the fungus; this is followed by clearance of the fungus, but over a longer period of time than the adjuvant-free vaccine of Example 1.

COMPARATIVE EXAMPLE 2

Subcutaneous Administration of *C. albicans*

The procedure of Example 1 was followed, except that the immunisations were carried out by sub-cutaneous injection. 10^{10} *C. albicans* cells in complete Freund's adjuvant were administered on day 0, 10^8 cells in incomplete Freund's adjuvant on day 14 and 10^8 cells on incomplete Freund's adjuvant on day 21. The results are shown in the following table.

	Day 35	Day 42	Day 49
Subcutaneous Vaccine	475	67	122

Again, subcutaneous administration resulted in an initial increase in the growth of the fungus, which was followed by clearance.

CLAIMS

1. An enteral non-adjuvanted vaccine comprising a killed fungal, bacterial or protozoal organism which infects the vagina.

2. A vaccine as claimed in claim 1, which is adapted for oral administration.

3. A vaccine as claimed in claim 1 or 2, wherein a unit dose contains from approximately 10^9 microorganisms to approximately 10^{13} microorganisms.

4. A vaccine as claimed in claim 1, 2 or 3, which contains killed *Candida albicans*.

5. A vaccine as claimed in claim 1, 2 or 3, which contains killed *Neisseria gonorrhoea*.

6. A vaccine as claimed in claim 1, 2 or 3, which contains killed *Gardnerella vaginalis*.

7. A vaccine as claimed in claim 1, 2 or 3, which contains killed *Trichomonas vaginalis*.

8. A process for the preparation of a vaccine as claimed in any one of claims 1 to 7, the process comprising admixing killed microorganism with a pharmaceutically or veterinarily acceptable carrier, filler, diluent and/or other adjunct.

9. The use of a killed microorganism which infects the vagina in the preparation of an enteral adjuvant-free vaccine.

10. A method of preventing vaginal infections in humans or non-human animals, the method comprising enterally administering a vaccine as claimed in any one of claims 1 to 7.

11. A method as claimed in claim 10, wherein the vaccine is administered at a time of high oestrogen levels.

12. A method as claimed in claim 11, wherein, in women not taking an oestrogen-based contraceptives, the vaccine is administered post-menstruation in the first half of the menstrual cycle.

13. A method as claimed in claim 11, wherein, in women who are taking an oestrogen-based contraceptives, the vaccine is administered at any time other than the week of their period.

14. A product comprising a vaccine as claimed in any one of claims 1 to 7 and a compound having oestrogenic activity for combined, simultaneous or sequential administration in the prevention of microbial infections of the vagina.

INTERNATIONAL SEARCH REPORT

International Application No.
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A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 39/00, 59/02, 39/002, 39/095

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K 39/-, CHEMICAL ABSTRACTS, MEDLINE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT WPAT: CHEMICAL ABSTRACTS CASM; MEDLINE A61K-039/1C; CANDIDA; GARDNERELLA; TRICHOMONAS; NEISSERIA () GONOR:; others

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,A, 2397839 (GRIMBERG) published 23 March 1979. See example 2 in particular	1-4, 8-9, 14
X	BE,A, 850847 (AMERICAN CYANAMID COMPANY) published 25 October 1976. See the examples in particular	1-3, 5, 8-14

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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INTERNATIONAL SEARCH REPORT

International Application No.
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C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Schlewinski, E et al. (1971) Orale Immunisierung mit nichtvermehrungsfähigen Mikroorganismen oder ihren Antigenen, Zbl Bakt Hyg., I Abt Orig A, (volume) 218, pages 93-104. See the whole document	1-4, 8-14
Y	US 4443431 (BUCHANAN et al.) published 17 april 1984. See column 7, last line in particular	1-2, 5, 8-10, 14
A	DD,A, 268868 (FORSCHUNGSINSTITUT FÜR LUNGENKRANKHEITEN UND TUBERKULOSE) published 14 June 1989	
A	WO,A, 86/05400 (AXON HEATHCARE LTD) published 25 September 1986	
A	US 4220638 (KARKHAUIS et al.) published 2 September 1980	
A	EP,A, 600396 (UNIVERSITY TECHNOLOGIES INTERNATIONAL INC) published 8 June 1994	
A	BE,A, 841068 (BACTEX, INC) published 25 October 1976	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/IB 95/00766

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
FR	2397839						
BE	850847						
US	4443431						
DD	268868						
WO	5605400	EP	214265	JP	62502193	GB	8506373
US	4220638	EG	14030				
EP	600396	CA	2109977	JP	7089872		
BE	841068						

END OF ANNEX

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EUR-CL (EPC): A61K039/00; A61K039/002, A61K039/02 , A61K039/095 , A61K039/245

ABSTRACT:

CHG DATE=19990617 STATUS=O>An enteral non-adjuvanted vaccine comprises a killed microorganism which infects the vagina. The microorganism may be a fungus, such as Candida albicans, a bacterium, such as Gardnerella vaginalis or Neisseria gonorrhoea, a protozoon, such as Trichomonas vaginalis, or a virus, such as Herpes genitalis. The absence of adjuvant gives a significant improvement in clearance of the microorganisms, compared to adjuvanted compositions.